ELECTROKINETIC STUDIES OF MARINE OVA. V. EFFECT OF PH-CHANGES ON THE SURFACE POTENTIALS OF SEA-URCHIN EGGS

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In a preliminary account (Dan, 1931), it was reported that the cataphoretic potentials of the unfertilized eggs of the sea-urchin, *Arbacia punctulata*, could not be reversed in sign on raising the concentration of hydrogen ions of the surrounding medium. The present paper reports experiments of a similar type which were repeated on other kinds of sea-urchins, *Anthocidaris crassispina* and *Pseudocentrotus depressus*, and the study was further extended to include fertilized eggs.

Method

Removal of the jelly. When the unfertilized surface was studied, unless otherwise stated, the jelly was removed by acid. While a dense suspension of eggs was being gently shaken, 1/10 N HCl was added drop by drop until the eggs began to show the first sign of sticking to the glass container. After a few more gentle shakings, a large quantity of sea water was added and the eggs were washed thoroughly by decantation. By this means, it was possible to reduce the time of the eggs' coming in contact with the concentrated acid to the minimum (less than 10 seconds).

pH adjustment. HCl was added to filtered sea water to an amount ample to break down the bicarbonate buffer system. After equilibration, more acid or alkali was added to adjust the pH value to the desired level by indicators. When the desired pH levels were near neutrality, 1/10 N acid or alkali was used, but when they were far removed from neutrality, ½ N solutions were added to minimize the lowering of the osmotic pressures. A slight lowering of the osmotic pressure by using 1/10 N within the limit here encountered does not affect the surface potentials of sea-urchin eggs (Dan, 1936). During the experimentation, the supernatant solution of the egg suspension was frequently checked for pH value to insure its constancy.

In very acidic media, the eggs were injured. At pH 3.5 or 4.0 they could still keep a fairly high fertilizability, if they were left unstirred in the acid and later fertilized after being returned to the normal medium. However, in the present experiment, stirring was technically unavoidable to some extent. In spite of this, pH values down to 2.0 were used for the purpose of obtaining a sufficient insight into the electrokinetic property of the egg surface, taking advantage of the fact that the killing of the eggs does not modify the potentials (Dan, 1934; 1936; see also Winslow, Falk and Caulfield, 1923; Abramson, 1929).

Measurement of the potential. The method of measuring the cataphoretic potentials was practically the same as was reported in the preliminary note and more fully discussed in the first paper of this series (Dan, 1933). This was to use a horizontal microscope on which a Northrop-Kunitz chamber was mounted. Since the stage of the horizontal microscope was situated vertically, the short axis of the cataphoretic chamber came to lie vertically. The chamber was filled with an egg suspension, and when the eggs began to settle, their paths of fall were recorded by a camera lucida. The focus of the microscope was previously adjusted to the level at $\frac{1}{5}$ of the depth of the chamber (Smoluchowski's layer) and only the eggs which came into sharp focus were selected for measurement. Two points were recorded to determine the path of fall; the electric current was then sent horizontally (i.e., at right angles to the path of fall) and the new path was traced. The deviation of the new path from the extrapolation of the initial path (with no current) corresponds to the cataphoretic movement. The interval of observation was measured by counting the beats of a metronome which was beating once in half a second. The potential drop within the chamber was measured by platinum poles inserted into the chamber through the glass wall by using a voltmeter with a high internal resistance (10,000 ohms, which is about 10 times the resistance of the chamber filled with sea water). The cataphoretic potentials were calculated by the formula $u = DH\zeta/4\pi\eta$, assuming that the dielectric constant of sea water is 80.

In order to minimize daily fluctuations in technique, readings were, as a rule, taken in three adjacent pH values on one day and different combinations of three pH's were studied each day. The data were later handled collectively according to the pH values.

Anthocidaris crassispina

The question, whether or not the surface potential is affected by fertilization, has been taken up by several investigators. Gray (1916) examined the agglutination of unfertilized and fertilized eggs of *Sphaerechinus* by $CeCl_3$ and came to the conclusion that fertilized eggs are carrying a more negative potential than unfertilized eggs, basing his conclusion on the fact that a more concentrated solution of cerium cations was required to agglutinate fertilized eggs than unfertilized ones. Runnström's observation (1929, p. 229) tends to support this conclusion. Vlès and Nouel in sea-urchin eggs (1922) and Fauré-Fremiet in the egg of *Sabellaria alveolata* (1924, p. 291) made observations on the behavior of these eggs in an electric field and arrived at the diametrically opposite conclusion, while Szent-Györgyi (1921) contended that sea-urchin eggs bore no charge.

However, some of the above investigations are based on indirect evidence, while the others are not quite free from technical dangers. Therefore, it was considered worth while to re-examine this point by a more direct technique. As a result, in the present paper, not only were the potentials on unfertilized and fertilized eggs compared in normal sea water, but the pH-potential curves of both were constructed through a wider range of the pH-scale. *Anthocidaris crassispina* was selected as one material. In the measurements of the fertilized eggs, the fertilization membrane was left intact. The perivitelline space of this species is very small, which is technically advantageous.

Data are summarized in Table I and graphically represented in Figure 1. (1) The potentials on both unfertilized and fertilized eggs of *Anthocidaris* cannot be

reversed in sign as far down as pH 2.0. (2) The maxima of both curves are found in the neighborhood of pH 7.0. (3) Though a statistical separation of the potentials on unfertilized and fertilized eggs at corresponding pH's is impossible, because of the wide fluctuation of the readings, the fact that the average values of the potentials on the fertilized eggs are invariably more positive than those of the unfertilized can be taken as a strong indication that the former are really more

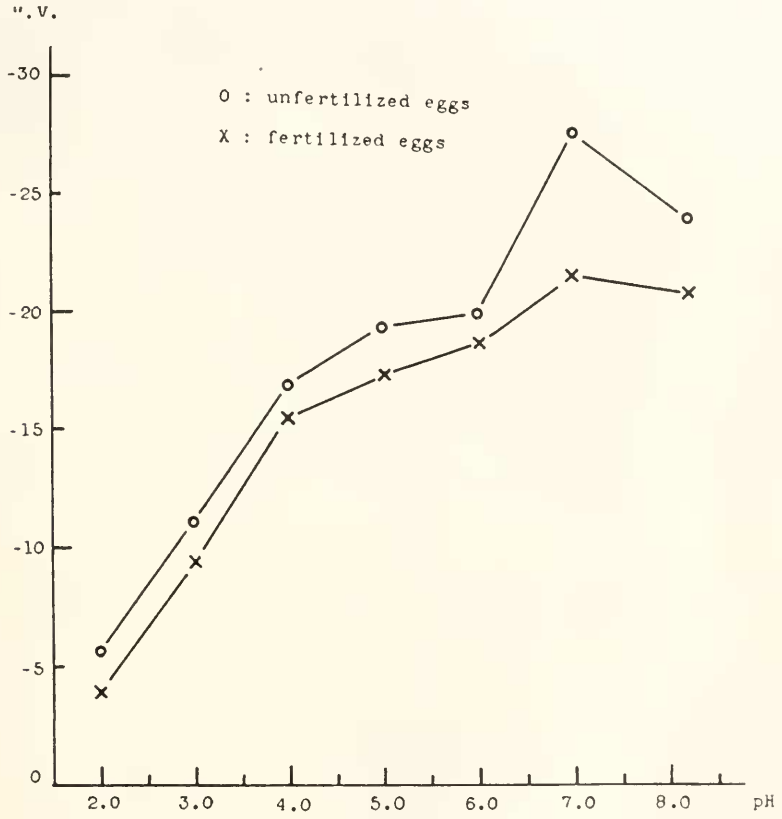

FIGURE 1. Anthocidaris crassispina.

positive. Needless to say, this is not due to an increase in the viscosity of the medium by the addition of the seminal fluid, since the eggs are later washed thoroughly. (4) The pH-potential curve for the unfertilized eggs is more zig-zag than that for the fertilized eggs, especially between pH 6 and 7. A repetition of the measurement in the following season (Series II of Table I) gave similar results which were incorporated in the final calculation.

PSEUDOCENTROTUS DEPRESSUS

In order to test the generality of the *Anthocidaris* findings, another sea-urchin *Pseudocentrotus depressus* was studied. However, in this case, attention was paid

TABLE I

Potentials on the egg surface of Anthocidaris crassispina *in millivolts with standard errors*

Medium	Unfertilized eggs without jelly			Fertilized eggs
	Series I	Series II	I and II combined	
Sea water	−24.9±0.78	−23.2±0.38	−23.9±0.34	−20.8±0.79
pH 7	−28.5±0.62	−26.3±0.57	−27.4±0.46	−21.5±0.58
pH 6	−19.9±0.71	−19.7±0.45	−19.9±0.35	−18.6±0.46
pH 5	−19.9±0.71	−18.5±0.43	−19.3±0.38	−17.2±0.61
pH 4	−13.3±0.62	−16.8±0.39		−15.5±0.47
pH 3	−11.0±0.59			− 9.4±0.48
pH 2	− 5.6±0.90			− 4.0±0.81
	Unfertilized eggs with jelly			
Sea water	−24.1±0.41			

chiefly to the unfertilized surface. pH intervals were taken to apparent 0.5 units of the indicators used. Data are summarized in Table II and Figure 2. As is evident from the figure, the general trend of the curve is very much the same as that of the two previous ones, with no reversal of the sign even in the most acidic medium (pH 2) and with a maximum slightly on the alkaline side of neutrality.

TABLE II

Potentials on the egg surface of Pseudocentrotus depressus *in millivolts with standard errors*

Unfertilized eggs without jelly

Medium	Potentials	Indicators
Sea water	−25.0±0.62	
pH 8.0	−24.4±0.55	Phenol red
pH 7.5	−27.0±0.69	
pH 7.0	−26.6±0.57	
pH 6.5	−24.5±0.53	
pH 6.0	−23.6±0.60	Brom cresol purple
pH 5.5	−22.0±0.44	
pH 5.0	−20.1±0.53	Brom cresol green
pH 4.5	−20.4±0.46	
pH 4.0	−16.8±0.43	
pH 3.5	−17.0±0.47	Brom phenol blue
pH 3.0	−13.8±0.55	
pH 2.5	−12.1±0.40	Meta cresol purple
pH 2.0	− 9.1±0.59	
	Fertilized eggs with jelly	
Sea water	−28.5±0.71	

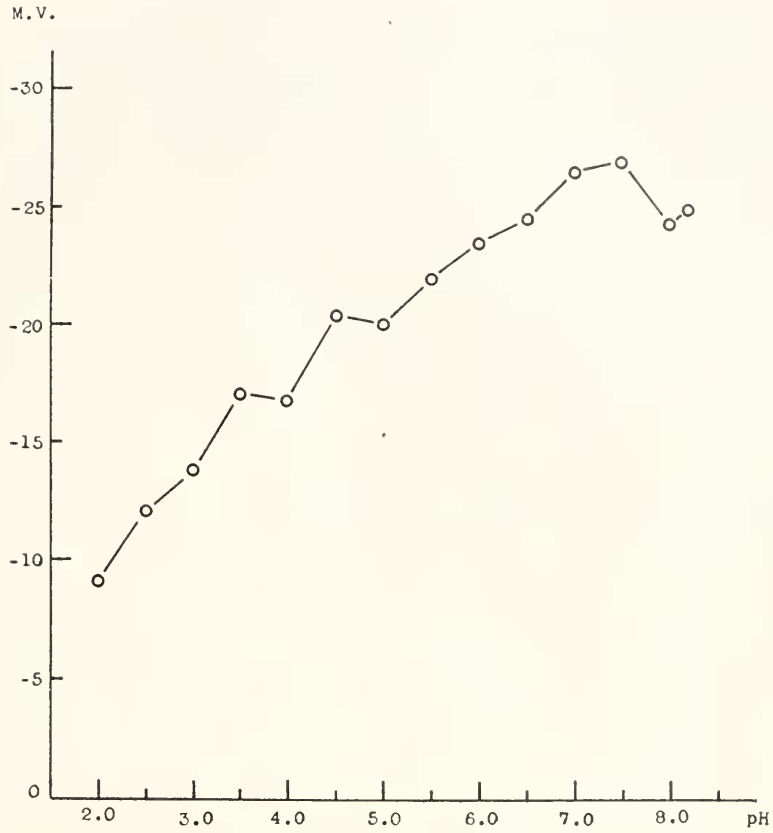

FIGURE 2. Pseudocentrotus depressus.

DISCUSSION

In the first paper of this series, it was reported that the potentials on the unfertilized and fertilized (with the fertilization membrane) eggs of *Arbacia punctulata* were − 30.3 and − 28.7 millivolts respectively and the potential on the unfertilized eggs in a suspension of dead sperm was − 26.7 millivolts. Comparing these figures with the sperm potential and its change under the influence of the egg water of the same species studied by Mudd, Mudd and Keltch (1929): the potential they found on the sperm was − 22.0 millivolts in sea water and "an increase in negative charge by 13 per cent followed on addition of the egg water." This means that the potential on the sperm in the egg water is − 24.9 millivolts. Now Mudd's experiment and the author's are reciprocal to each other. While the sperm potential which is − 22.0 millivolts changes to − 24.9 millivolts in the presence of the egg proteins, the egg potential which is − 30.3 millivolts at the beginning changes to − 26.7 millivolts in the presence of the sperm proteins. It is highly probable that the mixing of two kinds of proteins will shift the resultant potential somewhere in between the two extremities. This is particularly likely because both the seminal fluid in the author's experiment and the egg water used by Mudd et al. are ex-

tremely low in concentration. If a protein is added in high concentration, the potential will coincide with that of a pure protein.

The above postulate acquires a stronger support when the potential changes of other forms are considered. Mudd et al. studied not only the sperm potential of the sea-urchin (*Arbacia punctulata*) but also those of a starfish (*Asterias forbesii*) and a sand-dollar (*Echinarachnuis parma*) and further investigated their changes in egg waters in various combinations. Fortunately, the potentials of the eggs of these species have been measured by the author (Dan, 1934). As is shown in Table III, a sperm potential in egg water either falls between the potentials of the pure sperm and of the egg or coincides with one of them within 1 millivolt. In Mudd's data, the fact stands out as peculiar that the sea-urchin egg water has a specific power to cause a striking increase in the negative potential of any kind of sperm (see Table III, column 3) but this is now easily understandable because the egg protein of *Arbacia* has an exceptionally high negativity.

TABLE III

Comparison of egg potentials, sperm potentials and sperm potentials in egg water. As egg potential, the potential on unfertilized eggs without jelly is used. The figures in parentheses are the percentage changes in negativity of sperm potentials under the influence of egg waters.

Sperm potential \ Egg potential	Echinarachnius ca. −20.0 m.v.	Arbacia −30.3 m.v.	Asterias −19.9 m.v.
Echinarachnius −16.3 m.v.	−19.6 m.v. (+20%)	−21.7 m.v. (+33%)	−16.3 m.v. (0%)
Arbacia −22.0 m.v.	−21.6 m.v. (− 2%)	−24.9 m.v. (+13%)	−18.9 m.v. (−14%)
Asterias −18.1 m.v.	−17.7 m.v. (− 2%)	−21.7 m.v. (+20%)	−19.2 m.v. (+ 6%)

The present experiment was started with the above view in mind and it was hoped that the difference in the natures of the two proteins (if they are proteins) might be brought up more clearly by constructing pH-potential curves in a wider range. As a result, in the *Anthocidaris*-series, after fertilizing the eggs by the usual technique, excess of sperm fluid was added to secure a complete adsorption and then the eggs were thoroughly washed free from seminal fluid. In spite of this, both curves followed very similar courses except for the fact that the curve for the fertilized eggs consistently stayed below that of the unfertilized eggs.

It may be of some significance to point out in this connection that cholesterol has a very high electric mobility among substances investigated so far. According to Sugawara (1943a, 1943b) the fertilization membrane and its precursor of sea-urchin eggs are dissolved by proteolytic enzymes. This does not necessarily mean that the fertilization membrane and its precursor are made up solely of proteins. They are probably a compound of proteins and lipids and it is not impossible to imagine that one of the constituents predominates in the cataphoretic behavior. Further elucidation is greatly desired.

Concerning the course of the pH-potential curves, the absence of the isoelectric point and the existence of a maximum in the vicinity of pH 7 are worth noticing. Even though nothing definite can be based on the data at hand, these two points may be more or less connected with the high salt concentration of the medium, because cases are known in which the addition of salts either shifts the isoelectric point to the more acid side (Linderstrøm-Lang and Kodama, 1925; Sørensen and Sladek, 1929; Fauré-Fremiet and Nichita, 1927; Haffner, 1922) or brings the maximum around neutrality (Winslow, Falk and Caulfield, 1923). The absence of the isoelectric point here observed is contrary to Ashbel's finding (1931). Her paper states little about the technique of measurement. As far as this author's result is concerned, the pH-potential curves of sea-urchin eggs run quite close to those of *Bacillus cereus* in change of hydrogen ion concentration in concentrated salt solutions studied by Winslow, Falk and Caulfield (1923). The curves resemble each other not only in having no reversal of the sign in the acid range and having maxima near pH 7, but also in a decrease in the migration velocity (or the potential) in the alkaline region. This third similarity will be discussed briefly.

In the above-cited investigation of Winslow, Falk and Caulfield, *Bacillus cereus* loses its negative charge at about pH 10. In *Bacillus cereus* and *Bacillus coli,* the signs of the zeta potentials are reversed around pH 13.5 (Winslow and Shaughnessy, 1924). In the present data on sea-urchin eggs, since the pH scale is not extended so far into the alkaline region, only a downward trend is suspected. On the other hand, however, there is a great deal of indirect evidence from agglutination experiments, indicating that the absolute magnitude of the surface potential of sea-urchin eggs does decrease in the extremely alkaline region. Gray (1916) and Vlès (1924) concordantly found that the eggs agglutinate in a high pH region.

Naturally, a critical analysis is required before we can conclude that in sea-urchin eggs agglutination is really an indication that the absolute magnitude of the potential has decreased as it is in the case of bacteria or blood cells. This problem will be taken up in a later paper.

Summary

1. No isoelectric points were found in unfertilized eggs of *Pseudocentrotus depressus* and in both unfertilized and fertilized eggs (with fertilization membrane) of *Anthocidaris crassispina* when the pH was changed down to 2 in sea water.

2. In all the above cases, the maxima are found in the neighborhood of neutrality. Consequently, in natural sea water (pH, 8.2), the absolute magnitude of the potential is below the highest level.

3. Fertilized eggs carry a less negative potential than unfertilized eggs.

4. The data are discussed in connection with the sperm potentials and their changes in egg water reported by Mudd et al.

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